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Original Article

Hematological and histological overview of captive-bred female doublespotted queenfish *Scomberoides lysan* (Forsskål, 1775)

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Abstract: Health monitoring and assessment are required to support the improvement of maintenance efficiency of captive fish, but such information is lacking for the doublespotted queenfish *Scomberoides lysan* (Forsskål, 1775), a new candidate species for aquaculture in Thailand. The present study aimed to examine the morphological characteristics of erythrocytes and histopathological alterations in *S. lysan* females for the development of a health monitoring system. Female fish were randomly collected from a pond culture (n = 6, which was 34.5±0.9 cm in total length). Blood slides for hematological observations were prepared using the Wright-Giemsa stain method, while histological observations were made of tissues from the gills, kidneys, livers, and gonads. Blood smear test results showed that oval, mature erythrocytes were the most abundant cell type. Some erythrocytes exhibited nuclear morphological abnormalities, including kidney-shaped, polymorphic, and/or notched nuclei. The prevalence of such nuclei was only approximately 2%. The secondary lamellae of the gills displayed disorganization and aneurysms. Liver tissues displayed vacuolar degeneration of hepatocytes and blood congestion. Kidney tissues displayed tubular disorganization and renal degeneration. Melanomacrophage centers were present in large numbers in both liver and kidney tissues. Our analyses confirmed that the highest histological alteration index (HAI) calculated for liver tissue was a moderate alteration. The observations of *S. lysan* in captivity elucidated the cellular structure of this fish; the health of this fish might be reduced in captivity.

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Introduction

Assessing fish welfare aims to monitor the emergence or effects of captive environments by evaluating health in a location of interest. This assessment requires identifying a biomarker, which is a biological measure of the impact of environmental issues. The biomarker could be changes in organ weight and condition, or disorders of the blood and associated diseases (Auró de Ocampo and Ocampo, 1999). It is generally accepted that the health assessment of captive fish should use multiple biomarkers (Senarat et al., 2018). The examination of biomarkers, therefore, requires species-specific insights into histological and hematological processes. In the case of fish, hematological biomarkers are among the most efficient biomarkers of pathological alterations under inappropriate environmental conditions and captive conditions (Senarat et al., 2018). A strong evidence-backed knowledge base reported in wild fishes (Nussey et al., 1995), such as the occurrence of abnormal erythrocytes and micronuclei in fishes (Al-Sabti and Metcalfe, 1995), which are associated with erythrocytic chromosome damage and genotoxicity (Ayllon and Garcia-Vasquez, 2000). The association between changes in hematological and histological parameters and environmental problems was observed

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in an evaluation of the clinid *Labrisomus philippii* in Chile, where it lives in bodies of water contaminated with heavy metals (Montenegro and González, 2012). There is little literature describing hematological descriptions in captive fishes (Handbook, 2000; Proverbio et al., 2021).

Histological parameters were also cited as biomarkers of aquatic pollution in a review of marine biomonitoring (Au, 2004). Histological changes in various fish organs have been extensively discussed in studies of marine and riverine ecosystems (Lang et al., 2006) and anthropogenic pollution (Braunbeck et al., 1990; Schwaiger et al., 1997). A histological study identified decreased liver function in *Clarias gariepinus* from polluted water systems in North Africa (van Dyk et al., 2012), while a recently conducted health evaluation of *Hippocampus barbouri* from a hatchery revealed the stressful conditions of the aquaculture system with observations of liver and kidney histopathologies (Kamnurdnin et al., 2021). In a study of *Rastrelliger brachysoma* (Senarat et al., 2018), the deterioration of hepatic and renal tissues, as well as large depositions of melanomacrophages, were observed at the juvenile stage. The strong support given by Costa et al. (2009) for evaluating fish health in hatchery and field settings was not without good reason.

The doublespotted queenfish, *Scomberoides lysan* (Forsskål, 1775), is one of the important game fish in the family Carangidae. This marine species is also highly prized for dry fish production, and is now being considered as a candidate for aquaculture in Thailand. The aquaculture of this species has been successfully set up in rearing ponds by the Faculty of Science and Fisheries Technology on the Trang campus of Rajamangala University of Technology Srivijaya, Thailand. However, the health status of *S. lysan* in the aquaculture system has not yet been monitored, and it therefore remains unknown whether the aquaculture conditions are appropriate or not. The present study examines the erythrocytic characters and histological appearances for consideration as biomarkers of *S. lysan* in rearing ponds. The information obtained not only provides insight into the health status of the fish but also helps the future development of aquaculture for this species.

Materials and Methods

Fish collection and study areas: Six female *S. lysan* adults were randomly collected from the rearing ponds at the Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya, Trang campus. The detailed environmental conditions [water salinity, water temperature, pH, and dissolved oxygen (DO)] were recorded during the collection of the fish (September 2020). The fish were euthanized immediately by immersion in an ice–water bath (water and ice at a 1: 1 ratio) at 2-4°C (Wilson et al., 2009). The experiment was approved by the Animal Care and Use Committee of Rajamagala University of Technology Srivijaya (ID#IAC 13-12-64).

Analysis of blood cells: Blood samples were collected from the heart and tail base area of *S. lysan* using a 1 ml plastic syringe and a 21G needle (0.5 ml volume). About 10 µl of the blood sample was dropped onto a glass slide and spread into a thin film using another glass slide to prepare a blood smear by drying. Blood smear slides were fixed in methyl alcohol for 1 min and stained with Wright's Giemsa solution for approximately 15-20 min. The dye was rinsed off with PBS buffer (pH 7.8) for 5 min. The erythrocyte morphometry and nuclear abnormalities of erythrocytes (NAEs) from stained slides were analyzed under a light microscope (Leica 750). The percentage of NAEs was also calculated.

Morphometric and gravimetric analyses: All specimens were measured and weighed. Standard length (SL) was measured using a Vernier caliper to the nearest 0.01 cm, and total weight was measured using a digital scale to the nearest 1 g. After blood was drawn and gross morphology was observed, gills, kidneys, and livers were carefully resected and submerged in Ringer's solution. Morphological studies were conducted, and tissues were then placed in Davidson's fixative solution for further histological analyses.

Histological and histopathological observations: All collected tissues were stained with Harris's

Table 1. Classification of the severity of histological alterations in the gill, liver, and kidney of *Scomberoides lysan* (Poleksic and Mitrovic-Tutundzic, 1994; Paulo et al., 2012; Barbierl et al., 2019).

Histogical alterations (n = 6)	stages
Gill	
Disorganization of secondary lamellae	I
Lifting of lamellar epithelium	I
Presence of parasites	I
Complete lamellar fusion	II
Aneurysm	III
Liver	
Vacuolar degeneration of hepatocytes	I
Blood congestion	I
Melanomacrophage centers (MMCs)	I
Eosinophil granules in the cytoplasm	I
Pyknotic nuclei	II
Karyolysis of hepatocyte	III
Kidney	
Tubular disorganization	I
Renal degeneration	I
Eosinophil granules in the cytoplasm	I
MMCs	I
Karyolysis in renal cells	III

Table 2. Environmental water parameters in the rearing ponds of the captive-bred female doublespotted queenfish *Scomberoides lysan*.

Water physicochemical parameters		Duangsawasdi (1987)
Temperature	30.11±0.17	29.25-30.61
pH	7.60±0.10	7.74-8.18
Salinity	25.97±0.21	19.67-30.46
DO	9.05±0.90	5.63-6.98

hematoxylin and eosin (H&E). Histological tissue slides were prepared according to Presnell and Schreibman (1997). Selected organs were observed under a Leica digital 2000 light microscope (Heidelberg, Germany). Tissue samples that displayed histopathological lesions were used for further analysis.

Assessment of histological alteration index (HAI) and the average value of alteration (AVA): The severity of lesions in tissues of gill, liver, and kidney was assessed according to a semi-quantitative method using HAI (Poleksic and Mitrovic-Tutundzic, 1994) and the Average Value of Alteration (AVA) (Poleksic and Mitrovic-Tutundzic, 1994; Schwaiger et al., 1997). In accordance with HAI guidelines, alterations were assessed according to the standard criteria of progressive tissue damage stages (Table 1) (Poleksic and Mitrovic-Tutundzic, 1994; Paulo et al., 2012). The HAI was calculated using the following equation: $HAI = 1 \times \sum I + 10 \times \sum II + 100 \times \sum III$, where I, II, and III represent the alterations of stages I, II, and III, respectively. The HAI scores were then divided into five categories: 0 to 10 (normal organ/tissue functioning), 11 to 20 (slight alteration in the organ/tissue), 21 to 50 (moderate alteration in the organ/tissue), 51 to 100 (severe alteration in the organ/tissue) and values above 100 (irreparable alteration in the organ/tissue) (Poleksic and Mitrovic-Tutundzic, 1994). The AVA describes the degree of lesion severity and occurrence. Its range includes score 1 (no pathological alteration of organs), score 2 (slight or mild pathological alterations of organs), and score 3 (severe and extensive pathological alterations of organs) (Poleksic and Mitrovic-Tutundzic, 1994).

Results

Environmental factor measurement and morphometry: The water quality of the rearing ponds housing *S. lysan* was assessed from environmental measurements (Table 2). The average value of DO

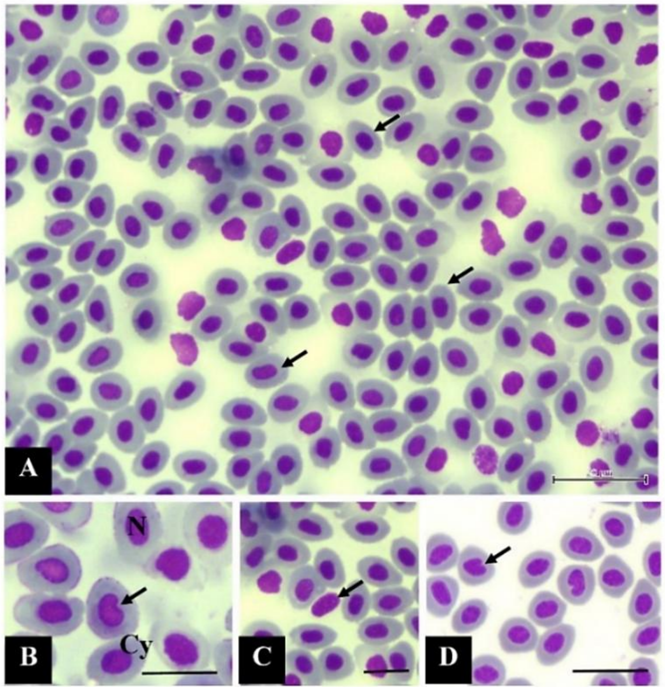

Figure 1. Light microscopy showing erythrocytes and nuclear abnormalities of female *Scomberoides lysan*. A: The overall blood smear shows erythrocytes (arrows). B: Erythrocytic nuclear abnormalities included kidney-shaped nuclei (arrow). C: The overall blood smear displayed thrombocytes (arrow). D: Nuclear abnormalities of erythrocytes included notched nuclei (arrow) (Scale bars B-D = 10 µm; Abbreviations: Cy = cytoplasm, N = nucleus).

was 9.05±0.90 mg/L, while the total length of this fish was 34.5±0.9 cm.

Hematological study and characteristics of erythrocytes and thrombocytes: Erythrocytes in blood smear slides of *S. lysan* were oval or elliptical (Figs. 1A-B). The nucleus and cytoplasm were stained dark purple and light pink, respectively (Fig. 1A). The measured morphological characteristics of erythrocytes included erythrocyte length (EL, 8.46±0.06 µm), erythrocyte width (EW, 5.95±0.05 µm), giving an EW-EL ratio of 1.42; nucleus length (NL, 4.16±0.04 µm) and nucleus width (NW, 2.79±0.02µm), giving an NL-NW ratio of 1.49. Thrombocytes were oval with an oval and basophilic nucleus (Fig. 1C). Erythrocytes from all *S. lysan* specimens showed morphological abnormalities, including kidney-shaped nuclei (Fig. 1B, 3% proportion) and notched nuclei (Fig. 1D, 1% proportion).

Histological and histopathological examination:

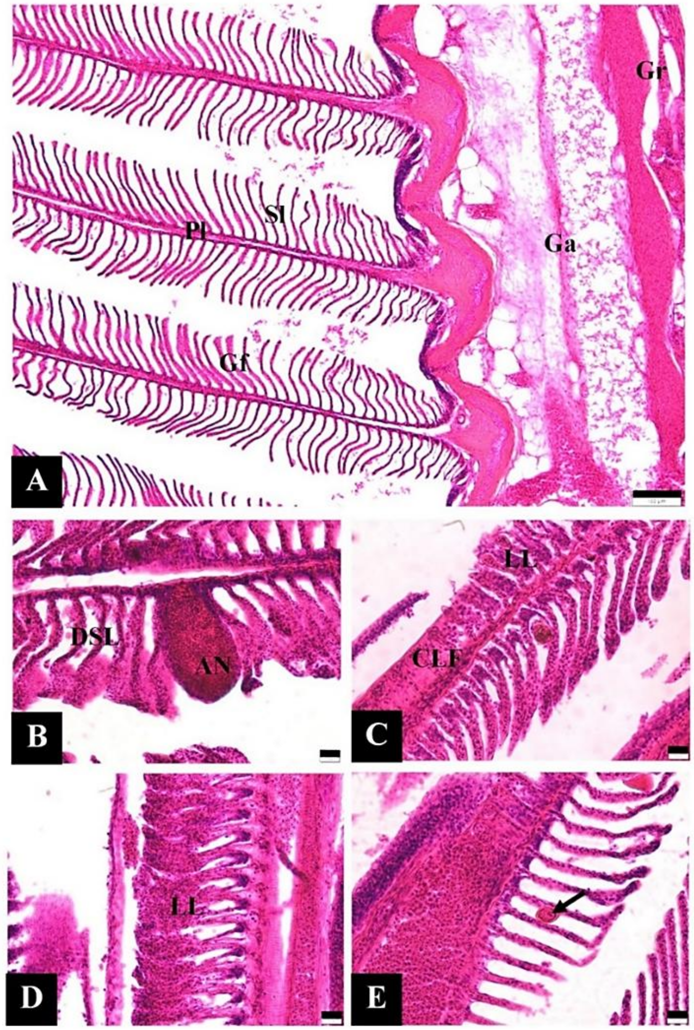

Figure 2. Light microscopy and histopathology of the gill of female *Scomberoides lysan*. A: The gill comprised the gill arch (Ga) and gill filament (Gf). B: Disorganization of the secondary lamellae (DSL) was observed together with aneurysms (AN). C-D: Lifting of lamellar epithelium (LL) and complete lamellar fusion (CLF)were present. E: Parasites were observed (arrow) (Scale bars A = 100 µm, B-E = 20 µm; Abbreviations: Pl = primary lamella, Sl = secondary lamella, and Gr = gill arch).

The gills of *S. lysan* had a comb-like structure consisting of gill rakers, gill arches and gill filaments (Fig. 2A). The gill filament was made up of primary and secondary lamellae (Fig. 2A). Several histopathological alterations were found, including disorganization of secondary lamellae (Fig. 2B), aneurysms (Fig. 1B), lifting of lamellar epithelium (Figs. 2C-D), complete lamellar fusion (Fig. 1C), and unidentified parasites (Fig. 1E).

The liver of *S. lysan* was enclosed within the hepatic capsule (Fig. 3A). The liver structure featured hepatic cords lined with hepatocytes, sinusoids, and

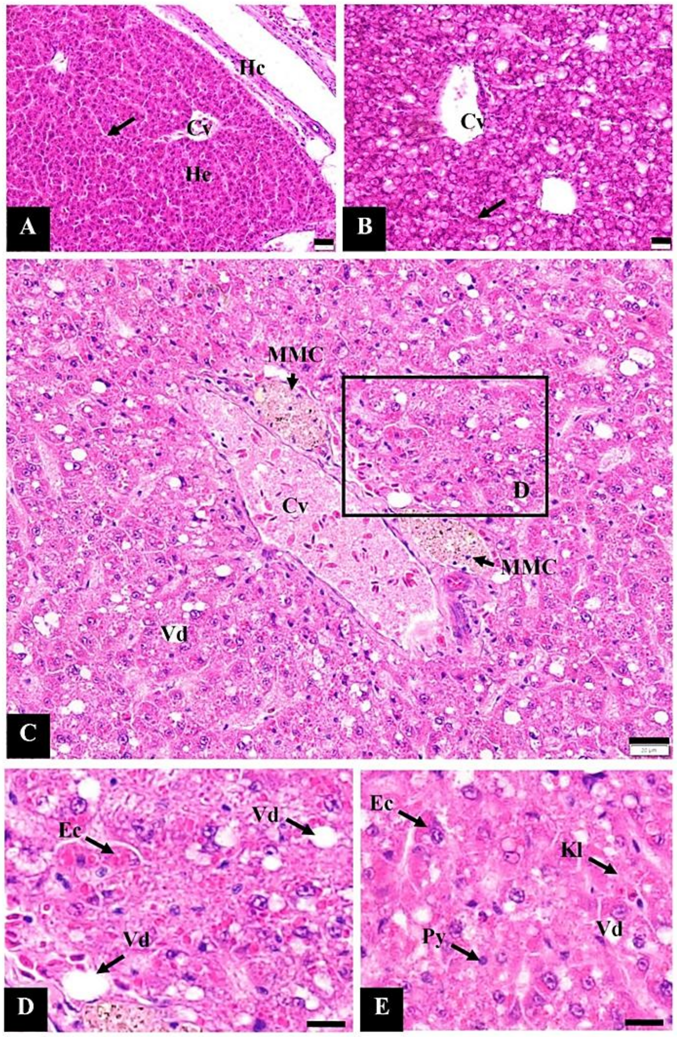

Figure 3. Light microscopy and histopathology of the liver of female *Scomberoides lysan*. A-B: Liver tissues displayed hepatocytes (Hc), the central vein (Cv) and hepatic sinusoids (arrows). C: Observed histological alterations included vacuolar degeneration of hepatocytes (Vd) and melanomacrophage centers (MMC). D-E: High-magnification images show eosinophil granules in the cytoplasm (Ec), vacuolar degeneration of hepatocytes (Vd), pyknotic nuclei (Py) and karyolysis of hepatocytes (Kl) (Scale bars A-C = 20 µm, E = 10 µm; Abbreviations: Hc = hepatic capsule).

the central vein (Figs. 3A-B). Identified histological alterations (Figs. 3C-E) included vacuolar degeneration of hepatocytes, eosinophil granules in the hepatic cytoplasm, blood congestion (data not shown), MMCs, pyknotic nuclei, and karyolysis of hepatocytes.

The kidney of *S. lysan* was elongated and contained the renal tubule, glomerulus, and hematopoietic tissue (Fig. 4A). Observed histological alterations to kidney tissue included tubular disorganization, renal

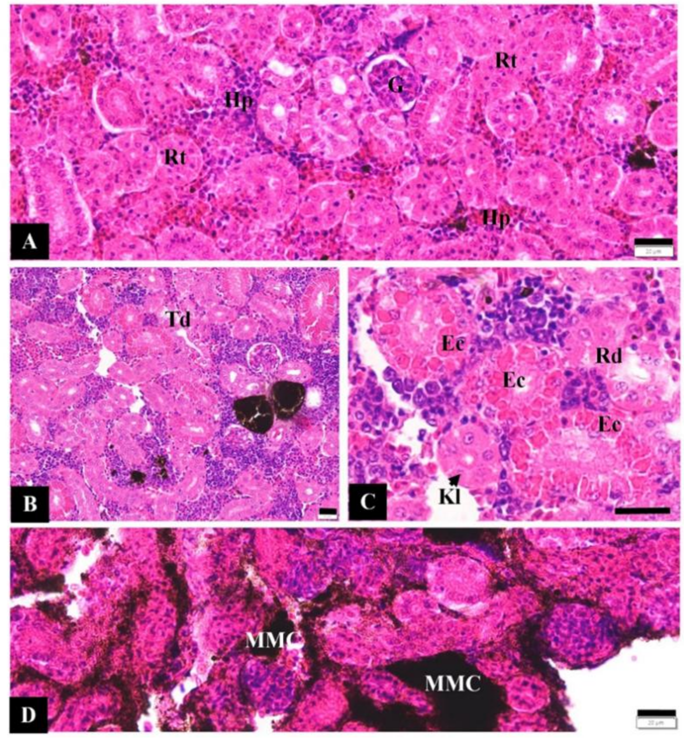

Figure 4. Light microscopy and histopathology of the kidney of female *Scomberoides lysan*. A: The kidney was an elongated organ and contained the renal tubule (Rt), glomerulus (G), and hematopoietic tissue (Hp). B-D: Various histological alterations were found, including tubular disorganization (Td), renal degeneration (Rd), eosinophil granules in cytoplasm (Ec), melanomacrophage centers (MMCs), and karyolysis in renal cells (Kl) (Scale bars A-D = 20 µm).

degeneration, eosinophil granules in the cytoplasm, MMCs, and karyolysis of renal cells (Figs. 4C-4F).

Semi-quantitative analysis: The present study conducted the HAI of *S. lysan*, in which moderate alterations were observed in all selected organs (gills (23.3%), liver (25%), and kidney (23.3%).

Discussions

Environmental factors were measured to assess the water quality of rearing ponds housing *S. lysan*. The salinity, pH, and water temperature fell within the range of standard values for freshwater resources (Duangsawasdi, 1987), indicating that the experimental fish were living in water of good quality.

The morphology of erythrocytes in *S. lysan* was comparable to that observed in *Schizothorax prenanti* (Fang et al., 2014) and other teleosts (Singkhanan et al., 2019). Erythrocytic morphology is related to metabolic diversity and aerobic swimming ability

(Lay and Baldwin, 1999), possibly being associated with the physiological adaptation of animals (Campbell and Murru, 1990). An increased volume of erythrocytes is required to increase oxygen transport and oxygen exchange rate (Lay and Baldwin, 1999). Our results suggest that *S. lysan* may require a higher amount of oxygen than other fishes (Lay and Baldwin, 1999). Thrombocytes are commonly found in Osteichthyes (Singkhanan et al., 2019), and their roles include clotting, platelet activation, and the production of inflammatory exudates with phagocytic activity (Stosik et al., 2019). The abnormalities of erythrocytes found in *S. lysan* included kidney-shaped and notched nuclei. These nuclear abnormalities may be caused by toxic substances, such as copper and cadmium (Summak et al., 2010), and/or genotoxic damage (Ali et al., 2008; Summak et al., 2010). These abnormalities may contribute to reduced respiratory function; a condition commonly observed in fish living in polluted waters or captive conditions. However, the nuclear abnormalities were found in only 2% of erythrocytes. The data indicate that the culture conditions were good.

The gill is a major organ for respiration, regulation of the acid-base balance, and excretion (Wilson and Laurent, 2002). Gills are directly exposed to environmental contamination and are particularly sensitive to toxicants. The gill structure of *S. lysan* was similar to structures found in other fishes (Wilson and Laurent, 2002). Some areas of this organ showed several histological alterations; edema and the detachment of the lamellar epithelium are the first signs commonly reported from fish exposed to xenobiotics (Thophon et al., 2003) and heavy metals (Nero et al., 2006). The edema and lifting of lamellar epithelium are considered defense mechanisms that increase the structural distance between the epithelium and contamination or poor environmental conditions (Sola et al., 1995). Alterations such as lamellar fusion, dilation of the marginal channel, and aneurysms are associated with the rupture of pillar cells caused by contamination (Martinez et al., 2004). Since the presence of severe gill lesions was observed in this study, the overall histological conditions reveal weakened gills in captive *S. lysan*, indicating diminished respiratory, excretory, and osmoregulatory functions.

The liver is an organ that plays a role in the uptake, biotransformation, and detoxification of foreign compounds (Thophon et al., 2003). Since hepatic lesions are a sign of water pollution (Simpson et al., 2000), our experimental fish may be exposed to environmental stressors (Monteiro et al., 2005). Vacuolar degeneration, also known as hepatocellular lipidosis, was observed in the present study, similar to that reported by Kamnurdnin et al. (2010). Hepatic lipidosis and cellular degeneration of hepatocytes are linked to liver damage, leading to the loss of liver function (Greenfield et al., 2008). Several factors have been reported to cause the formation of hepatic lipidosis, including chlorinated hydrocarbons and other pollutants (Hendricks et al., 1984) and PCBs (Teh et al., 1997). Mishra and Mohanty (2008) and Vinodhini and Narayanan (2009) reported that vacuolations of hepatocytes commonly occurred after exposure to various toxicants. Also, hydropic vacuolation was observed in *Pleuronectes americanus* living in chemically contaminated habitats (Moore et al., 1997). Nevertheless, liver histopathology is also associated with advanced age, low nutritional content of food, and/or other environmental factors. *Sparus aurata* fed various dietary lipid contents exhibited liver histopathologies (Caballero et al., 1999).

Renal tubular disorganization in *S. lysan* is a well-known histological alteration indicative of an acute condition, whereas the presence of small granules in the cytoplasm and hyaline degeneration indicates chronic damage to the renal tissue. Yildirim et al. (2006) reported that hyaline droplet formation results from tubular reabsorption of plasma protein as well as glomerular damage. Our observation revealed that hepatic lesions might be induced by hydrophilic contaminants and pollutants (Meinelt et al., 1997). It should be emphasized that renal tissues from this study might be employed as biomarkers to study the effects of contaminants in a laboratory setting. More research is needed to precisely assess the effects of contaminants, such as metals and other chemical pollutants, in captive settings. The relationship between histological damage and kidney tissue and its functions also requires further investigation.

The presence of MMCs, as observed in the current study, was consistent with the findings of previous studies by Agius and Roberts (2003) and Louiz et al. (2018). Fish exposed to infectious organisms, contaminants, or stress exhibit a high number of MMCs, which serve as a biomarker of fish health (Alvarez-Pellitero et al., 2007; Robert, 2012). This finding is consistent with prior studies, which can contribute to a better understanding of the dynamics of fish health in both the wild and captivity (Steinel and Bolnick, 2017). Here, we confirmed that the kidney is the most vulnerable organ to environmental stresses. Monitoring stress responses using renal cellular alterations could be a suitable subject for further investigation (Mansouri et al., 2017).

The HAI of *S. lysan* showed that the highest value was found in liver tissue; however, all selected organs showed moderate alterations in the organ/tissue, as per the criteria of Poleksic and Mitrovic-Tutundzic (1994). Our observations suggested that they are sensitive organs, which might be affected in their functions.

Conclusion

We shed new light on the blood cell biology of this species and observed various histological observations in the principal organs of *S. lysan* reared in captivity. We confirmed the occurrence of gill edema, hepatocellular lipidosis, and kidney degradation. However, to redress the effects of overharvesting and habitat degradation, effective raising and aquaculture of healthy stocks are required for conservation and fisheries management. Regular monitoring of water quality and provision of adequate nutrition in *S. lysan* aquaculture settings is essential and should be regularly examined to achieve effective captive breeding of this species.

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